CD203c expression on human basophils is associated with asthma exacerbation

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Background: CD203c is a basophil cell surface marker used to diagnose and monitor various allergic diseases, but its relationship to asthma is not clear.
Objective: We determined whether CD203c expression levels are associated with stable and exacerbated asthma.
Methods: We used flow cytometry to compare spontaneous expression levels of surface markers on basophils from patients with stable or exacerbated asthma and from healthy subjects. Longitudinal changes in these expression levels were measured after basophil stimulation by IgE-dependent or IgE-independent mechanisms and compared with patients' asthma status.
Results: Spontaneous expression levels of CD203c were significantly higher on basophils from patients with asthma exacerbation than patients with stable asthma or healthy subjects. In contrast, no differences in spontaneous expression levels of CD63 or CD69 were observed among the 3 groups. Anti-IgE–induced expression of CD203c significantly increased in basophils during asthma exacerbation ($P = .005$). Low concentrations of *Dermatophagoides pteronyssinus* or IL-3 induced higher expression levels of CD203c during asthma exacerbation than during clinical improvement; induction of CD203c expression by these antigens therefore correlates with asthma control. In the patients with clinical improvement, there was a correlation between spontaneous CD203c expression levels and the percent predicted values of FEV_1 ($r = -0.761$; $P = .022$).
Conclusion: Asthma exacerbation was accompanied by increased expression of CD203c on basophils that decreased significantly during remission. Basophil expression levels of CD203c might therefore be used to monitor asthma in patients.
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Key words: *Airway inflammation, asthma exacerbation, basophil activation, basophil surface marker, biomarker, CD203c*

Abbreviations used
ENO: Exhaled nitric oxide
MFI: Mean fluorescence intensity
PEF: Peak expiratory flow
PGD2: Prostaglandin D2
15R-MePGD2: 15(R)-methyl prostaglandin D2

Airway inflammation is an important feature of bronchial asthma. Various kinds of cells and their mediators are involved in the pathogenesis of asthma. Mast cells and basophils can have an important role in the development of allergic inflammation through IgE-initiated and chemokine-initiated mediator release, although eosinophils are the effector cells most often associated with airway inflammation in people with asthma. Basophils secrete 3 key classes of inflammatory mediators in allergic diseases: vasoactive amines, lipid metabolites, and cytokines. It has been reported that the number of basophils in the airways is higher in patients with asthma than in normal subjects[1] and that this number increases during asthma exacerbation.[2] It was also found that human basophils produce high levels of IL-4 in the lung after segmental allergen challenge.[3] Oosaki et al[4] reported that in patients with spontaneous asthma attacks, urinary excretions of eosinophil protein X, leukotriene E4, and 11-dehydrothromboxane B2 significantly increase during the attack and return to control levels in the improved phase. In addition, Nakano et al[5] showed that patients admitted to a hospital because of nonresolving asthma exacerbation had higher plasma levels of C3a, a potent proinflammatory mediator, than patients discharged immediately after the exacerbation or patients with stable asthma. However, the changes in each mediator and the relationship among mediators during asthma exacerbations have not been well documented. It is important to improve our understanding of the mechanisms of asthma exacerbation and develop more effective, specific therapies.

Various surface markers expressed on human basophils, such as CD63, CD69, and CD203c, have been used to demonstrate the role of basophils *in vivo* and *in vitro*.[6,7] CD203c is considered to be the most useful marker of basophil activation and differentiation. The functional role of CD203c in basophil differentiation and maturation is not clear. Upregulation of CD203c on the basophil cell surface is the result of an early FcεRI–mediated event and has a role in late, cytokine-mediated activation of basophils.[8] Because of the increased cell surface levels of CD203c in response to IgE-dependent stimuli, this protein is also a promising marker of activation that can be used in flow cytometry–based diagnosis of allergy. Flow cytometry–based basophil tests have been used to diagnose anaphylaxis,[7] chronic urticaria,[9] and other allergic diseases, as well as to confirm the sensitization of patients to food, drugs, or insects.[10] These tests appear therefore to be

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suitable for detecting immediate-type hypersensitivity and to monitor the success of hyposensitization protocols in patients with allergy.

On the basis of these studies, we propose that CD203c expression on basophils is involved in the pathogenesis of asthma exacerbation. However, changes in the CD203c expression levels on basophils have not been examined in patients with asthma. We investigated the relationship between CD203c expression and the pathological condition of asthma. We determined whether the CD203c expression on basophils increases in patients with asthma exacerbation and returns to control levels after improvement with treatment. In addition, we examined the extent of change in CD203c expression levels after *in vitro* stimulation via IgE-dependent and IgE-independent mechanisms.

METHODS

Subjects

We conducted a hospital-based, prospective study from August to December 2008 in 28 patients with asthma exacerbation. They were recruited from the emergency or outpatient department of the Pulmonology-Allergy Department at Sagamihara National Hospital. The diagnoses of asthma and asthma exacerbation were based on clinical history, symptoms, examination findings, and pulmonary function parameters, in accordance with international guidelines.[11] Inclusion criteria were an asthma history of at least 12 months, peak expiratory flow (PEF) of <60% of the normal predicted value, no smoking history, and meeting criteria for discharge after 120 minutes of therapy. Patients were excluded from this study if they had chronic obstructive pulmonary disease, definite bacterial airway infection, pneumonia, or autoimmune disease; needed immediate resuscitation or airway intervention; or had received systemic corticosteroids or allergen immunotherapy in the preceding 4 weeks. Nonresponders (basophils did not release histamine in response to anti-IgE or failed to upregulate CD203c) were also excluded. Sixteen patients with stable asthma without exacerbation in the preceding 3 months and 11 healthy control subjects were enrolled for comparative analyses. Sample size was calculated assuming a power of 0.8. Permission to conduct this study was obtained from the Ethics Committee of the Sagamihara National Hospital; all the participants gave their informed consent.

Study design

At the time of asthma exacerbation, all patients were given 2.5 mg salbutamol (Venetlin; GlaxoSmithKline, Tokyo, Japan) to inhale through a nebulizer and 125 mg methylprednisolone (Solu-medrol; Pfizer, Tokyo, Japan) intravenously. Subsequently, patients were given a single, daily, oral dose of 20 mg methylprednisolone (prednisolone; Shionogi, Osaka, Japan) for 5 days. All other asthma therapies were unchanged for each patient during the study. PEF was measured at baseline, after inhalation of the bronchodilator, 120 minutes after therapy, and on clinical improvement. Predicted values for PEF were based on data reported by Tsukioka.[12] Blood samples for flow cytometry were collected as soon as possible before systemic corticosteroid therapy.

Four weeks after asthma exacerbation it was confirmed that the patients met the definition of clinical improvement—that they no longer had symptoms, did not need reliever therapy, and had PEF of approximately 80% of the predicted values. All the patients were evaluated by chest radiograph and spirometry analyses, and exhaled nitric oxide (ENO) and blood samples were collected. Spirometry, including FEV_1, was carried out by using Minato Autospiro AS-303 (Osaka, Japan) in accordance with the American Thoracic Society criteria.[13] ENO was collected and measured in accordance with the recommendation of American Thoracic Society/European Respiratory Society (Sievers Instrument, Boulder, Colo).[14]

Reagents

Reagents used in the study included the allergenicity kit for quantification of basophil CD203c expression (Beckman Coulter, Fullerton, Calif); phycoerythrin-cyanine 7–conjugated anti-CD3 antibody; phycoerythrin-conjugated antibodies against CD63, CD69, and CD203c (Beckman Coulter); fluorescein isothiocyanate–conjugated anti-CRTH2 (Santa Cruz Biotechnology, Santa Cruz, Calif); recombinant human IL-3 (R&D Systems, Minneapolis, Minn); and 15(R)-15-methyl prostaglandin D2 (15R-MePGD2; Cayman Chemical, Ann Arbor, Mich). *Dermatophagoides pteronyssinus* 1 (Der p 1) was supplied by H. Yasueda at Sagamihara National Hospital, Sagamihara, Japan.[15]

Measurement of CD203c surface expression by flow cytometry

Boumiza et al[10] developed a 3-color flow-cytometric protocol to monitor basophil activation on the basis of identification of cells that are $CD3^-$, $CRTH2^+$, and $CD203c^+$; it was used to distinguish basophils from other cells in whole-blood samples. Expression of CD203c on basophils was analyzed by flow cytometry using the allergenicity kit according to the manufacturer's instructions. Briefly, after EDTA-containing peripheral blood (7 mL) was centrifuged at 260*g* for 10 minutes, the cells isolated from the buffy coat were incubated with serial dilutions of Der p 1, IL-3, 15R-MePGD2, or mouse monoclonal antihuman IgE for 15 minutes at 37°C in the dark after addition of a calcium solution, to override the chelating capacity of EDTA. Phycoerythrin-cyanine 7–conjugated anti-CD3, fluorescein isothiocyanate–conjugated anti-CRTH2, and phycoerythrin-conjugated anti-CD203c antibodies were also added during the reaction. The reactions were stopped, and erythrocytes were removed by adding the lysis solution included in the kit. After incubation, the cells were washed once with 2 mL PBS and fixed in 0.5 mL 0.1% formaldehyde. Expressions of CD63 and CD69 were analyzed in a manner similar to that of CD203c.

Samples were analyzed by using a FACSCalibur with CellQuest software (Becton Dickinson, Franklin Lakes, NJ). Basophils were detected on the basis of forward side scatter characteristics, lack of CD3 expression, and expression of CRTH2 (see this article's Fig E1). In each assay, an isotype control was used for negative staining. Data was expressed as net mean fluorescence intensity (MFI) (actual MFI – MFI of isotype IgG control).

Histamine release assay

To evaluate the IgE-dependent reactivity of basophils *in vitro*, parallel blood samples were collected from patients, incubated with heparin, and analyzed by a histamine release test, as previously described.[16]

Statistical analysis

Data were analyzed by using SPSS for MS Windows, version 12.0 (SPSS, Inc, Chicago, Ill). Data are presented as median values with ranges or mean values ± SEMs. Comparisons of paired data were carried out by using the Wilcoxon signed-rank test for calculation of differences. In all the other calculations, an unpaired *t* test or the Mann-Whitney test was used. When more than 2 groups were compared, Kruskal-Wallis ANOVA followed by the Mann-Whitney test with Bonferroni correction or the Friedman repeated-measures ANOVA followed by the Wilcoxon *t* test with Bonferroni correction was carried out. Correlation was evaluated by the Spearman rank test. Differences were considered significant if P values were less than .05.

RESULTS

Patient characteristics

Background data from the subjects enrolled in this study are summarized in Table I. Among the 28 patients with asthma exacerbation, 4 were excluded: 2 had bacterial pneumonia, 1 had eosinophilic pneumonia, and 1 had prolonged asthma exacerbation after routine therapy. None were excluded based on failed upregulation of CD203c expression. In total, 24 patients with asthma exacerbation, 16 patients with stable asthma, and 11 healthy control